UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Marketing Service

METHODS OF CHEMICAL ANALYSIS AND GRADE CALCULATIONS FOR COTTONSEED

FOR LICENSED COTTONSEED CHEMISTS

Approved by the Chief,
Agricultural Marketing Service
July 5, 1939

Superseding Methods Approved
June 7, 1938

Washington, D. C.
July 1939

COTTONSEED

METHODS OF CHEMICAL ANALYSIS AND GRADE CALCULATIONS APPROVED BY THE CHIEF, AGRICULTURAL MARKETING SERVICE, JUNE 5, 1939

SECTION 1. Methods for the Analysis of Cottonseed.

(a) Laboratory Sample. The sample received at the laboratory shall consist of 2 pounds of cleaned seed or sufficient to fill a container of 155 cu. inches capacity. It shall be sealed in an air-tight container and shall be accompanied by a certificate by a licensed cottonseed sampler in the form approved by the Chief, Agricultural Marketing Service, U. S. Department of Agriculture.

(b) Handling of Sample. The sample shall be examined by the chemist who shall correct the weights reported to him by the sampler, for such additional foreign matter as he may find in the sample by passing it over a 6-mesh screen and picking out all other particles of foreign matter. The sample shall then be placed in an approved mechanical mixer and handled according to directions for the use of the machine (MacLellan Mixer No. 00-S is approved). The cleaned and mixed sample shall then be quartered and one-half of it returned to the original can and retained as a referee sample. The second half shall be preserved in an air-tight container and used by the chemist for his analysis.

SECTION 2. Original Moisture.

Determination. Weigh as rapidly as possible 2 samples of 8 to 10 grams each of the whole seed into shallow moisture dishes and distribute evenly. The uncovered dish containing the sample is placed in the oven specified in Section 3 (a) at 101° C. for from 12 to 16 hours, or most conveniently over night. The dish when removed from the oven is covered, cooled in an efficient desiccator and weighed, the loss in weight being calculated as moisture.

SECTION 3. Fuming and Grinding.

(a) Apparatus. A forced-draft circulatory oven approved by the A. O. C. S.

Fuming Oven. A double-walled tank constructed of sheet iron or copper, seams welded or brazed, and with the inner compartment open at the top. The compartment should be approximately 7¼ inches deep and 7 inches wide, to hold 2 rows of porous fuming pots. The oven may be of any convenient length, depending upon the number of seed pots to be fumed at a time. (For full description see appendix.)

Fuming Pots. A porous earthenware vessel, such as a 3-inch flower pot. (The unglazed, porous clay pots made for the purpose by the Niloak Pottery Company of Benton, Arkansas, have proved satisfactory.)

Grinding Mill. Bauer Bros. No. 148 Laboratory Mill, using No. 6912 plate. Speed of 3600 R.P.M. recommended.

(b) Preparation of Seed for Oil and Ammonia Determinations. Dry an approximately 60-gram portion, for 2 hours at 130° C. ± in an approved type of forced-draft circulatory oven. Toward the end of this drying period absorb into the inner walls and bottom of a porous earthenware vessel 1.5 c.c. of concentrated hydrochloric acid. The acid is distributed all over the side of the pot, and when absorbed, the inside of the pot must appear dry, otherwise a new pot must be substituted. Place the dried seed in the pot, cover and place in the fuming oven previously opened and ventilated for at least 5 to 10 minutes, and fume for 1 hour. The oven temperature should gradually rise to, but not exceed, 115° C. The lint should be loose and brittle, not scorched. Grind the sample in Bauer mill which has been adjusted to produce a fine meal. After grinding, open up the mill and carefully brush out all remaining ground seed onto a sizable smooth sheet of paper. It is important that the top of the hopper of the Bauer mill be fitted with a cover to prevent loss of seed during grinding. There should be practically no loss of material in grinding and if more than 1 gram of material is lost the whole process should be repeated as the lost material is not necessarily representative of the whole.

Mix the ground sample thoroughly. An approved method for doing this is by placing the ground material in a 1/2-gallon Mason fruit jar, together with a large rubber stopper. Replace the cover and shake violently until the ground material is thoroughly mixed, then transfer to a well-stoppered bottle or container, of just sufficient size to hold the material tightly so as to prevent percolation or vertical segregation of the components.

SECTION 4. Second Moisture.

Determination. Weigh 5 grams of the fumed and ground sample into moisture dish and dry at 101° C. for 2 hours in oven specified in Section 3 (a). Place cover on dish, cool in desiccator and reweigh. Calculate loss in weight as percent of moisture of fumed sample.

SECTION 5. Oil.

(a) Apparatus and Reagents. Extraction apparatus of Butt type. Allihn condenser with 12-inch jackets, fitted with cork

connections, is recommended. Petrolic ether of the following specification:

Initial boiling temperature -	Not less than 35° C.
Initial boiling temperature -	Not over 38° C.
Dry flask end point -	Not over 60° C.
Dry flask end point -	Not less than 52° C.
At least 95% distilling under	54° C.
Not over 60% distilling under	40° C.
Specific Gravity at 60° F., -	0.630 to 0.660
Color - - - - - - - - - - - -	Water white
Evaporation residue 100 cc -	Not over .0011 grams
Doctor test - - - - -	Sweet
Copper Strip Corrosion Test -	Non corrosive
Unsaturated compounds - -	Trace only permitted
Residue in distilling flask -	Neutral to methyl orange
Blotter Strip Odor Test - -	Odorless within 12 minutes
Aromatic compounds - - -	No nitro benzene odor
Saponification value - -	Less than 1.0 mg. KOH per 100 cc.

Distillation test to be made according to A.S.T.M. D 216-32. As a check on the evaporation residue, 250 cc of the petroleum ether and 0.25 g. of stearin or other hard fat (previously brought to constant weight by heating) when dried as in the actual determination shall not show an increase in weight exceeding 0.003 g. Copper strip corrosion test is made by inserting a small polished copper strip into the petroleum ether in the distilling flask. There should be no appreciable darkening of the copper.

Unsaturated compounds shall be determined by the method for determining olefins on page 154 of the March 15, 1938, Analytical Edition of Industrial and Engineering Chemistry. Odor Test: Immerse one inch of a strip of white, unglazed blotting paper, approximately 1" x 4" x 0.166" in size, in the petroleum ether for 30 seconds, remove strip and allow to dry at room temperature in still air for 12 minutes. Aromatic Compounds: Add 5 drops of petroleum ether to 40 drops of concentrated sulphuric acid in a test tube, warm for 10 minutes, allow to cool for one-half hour, transfer to a shallow dish and dilute with water.

(b) Determination. Weigh accurately duplicate samples of 4 to 5 grams of the fumed and ground seed, wrap in a 150 mm. filter paper (S & S #597, or equivalent grade) and rewrap in a second paper or papers in such manner as to prevent escape of the meal, leaving the top of the second paper open like a thimble. A piece of absorbent

cotton may be placed in the top of the thimble to distribute the dropping ether if preferred. Place 25 cc. of petrolic ether in a tared flask of 125 cc. capacity and extract sample for 4 hours. The ether should drop on the center of the thimble at a rate of at least 150 drops per minute. The volume of the solvent should be kept approximately constant. The solvent is evaporated off until no trace remains, cool to room temperature, and weigh. The last traces of ether are sometimes difficult to detect by odor and in case of doubt evaporate for an hour or longer until constant weight is obtained. Calculate the oil content as shown in the following example:

EXAMPLE OF CALCULATION

Petrolic ether extract	1.025 Grams
Original moisture plus total foreign matter up to and including 1% 12.2% + .8% =	13.0%
Second moisture	2.6%
Total foreign matter up to and including 1%.	.8%
Weight of sample	5.00 Grams

$$\text{Percent Oil} = \frac{1.025}{5} \times \frac{87}{97.4} = 18.3$$

SECTION 6. Ammonia Determination.

(a) Apparatus and Reagents. Use Kjeldahl digestion flasks of 650 c.c. or 800 c.c. capacity, digestion rack for supporting flasks over burners, distillation stand with condensers, flasks for receiving the ammonia distillate, metallic mercury or mercuric oxide, sodium or potassium sulphate, concentrated sulphuric acid, zinc (preferable granular 20 mesh), 4 percent solution of potassium or sodium sulphide, and caustic soda solution (specific gravity of 1.50).

(b) Digestion Procedure. Digest 1.7034 or 1.401 grams of the sample in a Kjeldahl flask with approximately 0.5 gram metallic mercury or 0.7 gram mercuric oxide, 10 grams of sodium or potassium sulphate, and 25 c.c. of sulphuric acid (specific gravity 1.84). Place the flask in an inclined position and heat below the boiling point of the acid from 5 to 15 minutes, or until frothing has ceased. Increase the temperature and continue digestion until the liquid becomes colorless, or until complete digestion is obtained. The process is the same from this point on as in the regular Kjeldahl method, except that no potassium permanganate is added.

(c) Distillation. After cooling, add about 300 c.c. of distilled water, a few granules of zinc to keep the contents from bumping, and 25 c.c. of a 4 percent solution of potassium or sodium sulphide, or a sufficient quantity to precipitate all the mercury. After mixing thoroughly, add 60 c.c. of a caustic soda solution (specific gravity 1.50), or sufficient to make strongly alkaline, pouring the solution down the side of the flask so that it does not mix at once with the acid solution. Connect the flask with a condenser of block tin, mix the contents of the flask by shaking, and distill into an accurately measured quantity of standard sulphuric acid solution (0.5N recommended) to which has been added 50 c.c. of distilled water, until at least 200 c.c. of distillate is obtained, taking care that the delivery tube reaches below the level of the standard acid. Add about 1 c.c. of a 0.2 percent aqueous solution of sodium alizarin sulphonate as the indicator. Either cochineal or methyl red may be used also as the indicator; with methyl red the solution may be titrated hot. Then titrate the distillate with a standard fixed alkali solution (a 0.25N sodium hydroxide solution is recommended).

(d) Blank Correction. Make blank test on all reagents and correct the titration of the above distillate accordingly.

(e) If the ammonia percentage found in the fumed, ground sample is found to be less than 3.70% or more than 4.50%, a second determination shall be made and if these two determinations do not agree within 1/10 of 1%, two additional determinations shall be made, the average of the two or three determinations agreeing most closely to be used in the calculation.

CALCULATION FOR AMMONIA

Example:

Quantity of 0.5N H_2SO_4 measured into flask	10.00 c.c.
Quantity of 0.5N H_2SO_4 for blank test on reagents	.06 c.c.
Quantity of 0.25N $NaOH$ used in titration	2.68 c.c.

$$\frac{10-0.06}{2} - \frac{2.68}{4} = 4.30 \text{ percent ammonia in fumed seed}$$

Original moisture	8.1
Foreign matter, up to 1.0	.9
Moisture in fumed seed	2.0

$$\frac{4.30 \times 0.91}{0.98} = 3.99 \text{ percent ammonia in original seed}$$

SECTION 7. Free Fatty Acid.

Determination. Dry 200 g. of the original clean sample of seed for not less than 30 to 40 minutes at a temperature of 100° - 105° C., and cool. Pass the cooled seed through a laboratory huller approved by the A. O. C. S. Separate the meats from the hulls by the use of a 4-6 mesh screen. Grind the meats in a Russwin No. 1 food chopper equipped with 16-tooth blade. Thoroughly mix sample. Proper grinding and complete separation of meats from hulls are essential points in obtaining concordant results. Without undue loss of time quarter the thoroughly mixed ground meats so as to obtain at least a 40-gram sample. Extract this sample by cold percolation in the following manner: Place the lower disc from a Knorr Extraction Apparatus in a Butt tube and place on it a layer of asbestos fibre suspended in petrolic ether. A satisfactory mat should allow none of the meats to pass through, but should allow the extracting solvent to flow through at about 150 drops per minute. Place the sample in the prepared tube, and add 50 cc. of petrolic ether followed by 2 portions of 25 cc. each of petrolic ether, each portion being allowed to flow through before the following portion is added. Allow the extracted oil to remain on the steam bath for 1½ hours to completely remove all trace of the solvent. Weigh 7.05 g. of the oil into a titrating flask, add 30 cc. of neutralized alcohol (S.D. Formula 30) or Isopropyl alcohol, 1 cc. of 1 percent phenolphthalein (10 cc. of petrolic ether may be added if necessary) and titrate the free fatty acid of the oil with standard 0.25 N. alkali. The flask is shaken vigorously during the titration, the end point being taken when a permanent pink is obtained which persists for at least one minute.

$$\text{Percent F.F.A.} = \frac{28.2 \times \text{normality of alkali} \times \text{cc. used}}{\text{weight of oil}}$$

If results indicate a free fatty acids content of 4 percent or higher the complete test should be duplicated.

SECTION 8. Calculation of Analysis.

(a) Data on reports of seed analyses should be expressed as follows:

Foreign Matter to	.1%
Oil to	.1%
Ammonia to	.01%
Free Fatty Acid, when 5% or under, to	.1%
Free Fatty Acid, when over 5%, to	.5%
Quantity Index to	.01%
Quality Index to	.1%

Grade to whole or half units, whichever the actual calculation is nearest.

Yields to whole units.

(b) From the moisture determined on the seed as received plus Total Foreign Matter up to and including 1 percent and the moisture determined on the fumed and ground sample, the figures for oil and ammonia are calculated back to the original basis as received, by the following formula:

M = Moisture in original seed.
FM = Total Foreign Matter up to and including 1 percent.
P = Moisture in fumed and ground sample.
F = Factor to multiply by to reduce to original basis as received.
(100 - [M + FM]) divided by (100-P) = F.

EXAMPLE:

Percent moisture in original seed	12.2
Percent total foreign matter up to and including 1 percent ...	.8
Percent oil in fumed, ground seed	20.5
Percent ammonia in fumed, ground seed	3.90
Percent moisture in fumed, ground seed	2.6
(100 - [12.2 + .8]) divided by (100-2.6)	89.32
F (factor)	89.32
89.32 x 20.5	18.3
89.32 x 3.90	3.48

SECTION 9.

(a) All calculations shall be carried out to the third decimal place.

(b) Fractions of exactly one-half shall be dropped if the next higher decimal figure is an even number and used to raise the next higher decimal figure if it is an odd number.

(c) The percentages of oil, moisture, and foreign matter shall be reported to one decimal place.

(d) The percentage of free fatty acids shall be reported in tenths of one percent when less than 5.0 percent; when more than 5.0 percent it should be reported in the nearest 1/2 percent.

(e) The quantity index, and the percentage of ammonia shall be calculated to two decimal places.

(f) Grade shall be reported as the nearest whole number or the nearest half number, whichever the actual calculation is nearest; that is to say, fractions up to and including 0.25 and more than 0.74

shall be reported as whole number grades, and those fractions between 0.26 and 0.74 inclusive shall be reported as half number grades.

(g) A sample certified by a licensed sampler as hot or fermented shall not be designated as "Off Quality" unless the chemist shall find evidence of damage due to fermentation or heating.

(h) No certificate of grade shall be issued until after the lapse of 20 hours after receipt of the sample by the chemist.

(i) The following form of grade certificate is approved. The certificate shall not contain any advertising matter.

U.S.
(SEAL)
D.A.

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Marketing Service
COTTONSEED GRADE CERTIFICATE
A. B. C. LABORATORIES cooperating
30 Wall St., New York City

Issued at _____

Date _____

Submitted by _____

_____

Identified as _____

Condition reported by sampler _____

Point of origin _____ County _____ State

Sample certificated by _____
Licensed Cottonseed Sampler No. _____

FOREIGN MATTER

Calc. from Sampler's Report _____% Oil _____%

Add'l Found by Laboratory _____% Ammonia _____%

Total _____% Free Fatty Acid in Oil _____%

QUALITY INDEX DEDUCTIONS

Moisture _____%

Acct. excess Foreign Matter _____

GRADE CALCULATION

Acct. excess Moisture _____ Quantity Index _____

Acct. excess F.F.A. _____ Quality Index _____

Total _____ GRADE _____

ANALYSIS, (Basis foreign matter content not exceeding one percent)

I hereby certify that the above analysis was made according to the laboratory methods approved by the Chief of the Agricultural Marketing Service and that the grade given is according to the official standards of the United States.

Certificate No. _____ _____
Licensed Cottonseed Chemist No.

Following data for information only -- not a part of the Standard Grades.

Appendix

Fuming Oven or Tank

The double-walled jacket should surround the oven on four sides and bottom; the space between walls should be about $1\frac{5}{4}$ inches. The jacket should be provided with a breather pipe consisting of a 3/4-inch close nipple welded into the end of the tank near the top, with elbow and 10-inch nipple extending vertically and a filler opening. The jacket should be filled with vegetable or mineral oil which can be heated to a temperature of about 175° C. A nipple should also be provided through which a thermometer can be inserted into the oil. Flat bottom trays of perforated metal, with handles which will fit within the compartment should be provided to hold the pots (conveniently ten to the tray) and enable them to be easily removed. Small angle irons should be welded on the bottom of the compartment so that the trays will not rest directly on it, thus permitting circulation of heated air around the pots.

If the oven is made of a length to accommodate more than one tray of ten pots, sheet iron partitions should be firmly attached between the sections for trays, so that one tray may be removed without interference with another. A compartment $17\frac{1}{2}$ inches long will accommodate ten pots in a double row of five.

A lid of sheet metal with handle should be provided to cover the inner compartment, lugs should be attached on the under surface to center the lid in place. One lid for each ten pots section is preferable. Holes for ventilation and for a thermometer should be cut in the lid, two (2) one (1) inch diameter ventilating holes for each ten pots capacity.

This oven or fuming tank may be supported in any convenient manner and heated either with gas burners or with electric space heaters placed in contact with the bottom. Thermostatic regulation is not essential but may be convenient.

(NOTE: Sheet iron painted with an aluminum lacquer paint has been found to resist the corrosive effect of the hydrochloric acid.)

Examples of Grade Calculations

Base analysis — No. 1. Analysis: 18.5% oil, 3.50% NH_3, 12.0% H_2O, 1.8% F.F.A., 3.0% F.M.

4 x 18.5 =	74.0
6 x 3.50 =	21.00
Plus	5.0
Quantity Index	100.00
Quality Index	100.0

$$\frac{100 \times 100}{100} = 100 = \text{Grade } 100.0$$

Superior Quality — No. 2. Analysis: 18.6% oil, 3.94% NH_3, 9.5% H_2O, 0.5% F.F.A., 0.5% F.M.

4 x 18.6 =	74.4
6 x 3.94 =	23.64
Plus	5.0
Quantity Index	103.04
Quality Index	102.0

$$\frac{103.04 \times 102}{100} = 105.10 = \text{Grade } 105.0$$

Oil Below 17% — No. 3. Analysis: 16.5% oil, 4.10% NH_3, 7.50% H_2O, 0.3% F.F.A., 0.5% F.M.

5 x 16.5 =	82.5
6 x 4.10 =	24.60
Minus	12.0
Quantity Index	95.10
Quality Index	100.0

$$\frac{95.10 \times 100}{100} = 95.1 = \text{Grade } 95.0$$

Subquality, high moisture and F.F.A.

No. 4. Analysis: 19.0% oil, 3.84% NH_3, 13.5% H_2O, 2.4% F.F.A., 3.0% F.M.

4 x 19.0 = 76.0
6 x 3.84 = 23.04
Plus 5.0

Quantity Index 104.04

Quality Index reduced by:

3.0 units acct. F.F.A.
1.5 units acct. H_2O
4.5 Total
95.5 Quality Index

$$\frac{104.04 \times 95.5}{100} = 99.36 = \text{Grade } 99.5$$

Off Quality Free Fatty Acids above 12.0%

No. 5. Analysis: 19.5% oil, 3.84% NH_3, 12.0% H_2O, 15.5% F.F.A., 1.0% F.M.

Off Quality Grade = 33.5

Below Grade Cottonseed

No. 6. Analysis: 19.0% oil, 3.80% NH_3, 14.0% H_2O, 16.8% F.F.A., 1.0% F.M.

4 x 19.0 = 76.0
6 x 3.80 = 22.80
Plus 5.0

Quantity Index 103.80

Quality Index reduced by:

2.0 units acct. H_2O
75.0 units acct. F.F.A.

Below Grade Cottonseed.